OncoTargets and Therapy

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ORIGINAL RESEARCH

Salivary expression of soluble HER2 in breast cancer patients with positive and negative HER2 status

This article was published in the following Dove Press journal:
OncoTargets and Therapy
14 July 2014
Number of times this article has been viewed

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Background: The aim of this study was to investigate the relationship between salivary concentration of the soluble fragment of the HER2 (human epidermal growth factor receptor) protein and its status in mammary tissues.

Methods: This case-control study was done in 27 breast cancer patients with no visible metastatic disease treated at the gynecology service, Maternity Souissi Hospital, Rabat, Morocco. Two groups were selected, ie, patients with positive and negative HER2 status in mammary tissue. The salivary HER2 protein concentration was assessed by enzyme-linked immunosorbent assay. The salivary HER2 concentration was compared between the HER2-positive and HER2-negative groups using the Mann-Whitney *U* test. A *P*-value <0.05 was considered to be statistically significant.

Results: No statistically significant difference in salivary HER2 protein expression was found between the case and control groups. There was also no significant difference in clinical characteristics according to positive and negative HER2 status ($P>0.05$), except for the progesterone hormone receptor which was statistically significant in both the case and control groups ($P=0.047$).

Conclusion: According to our data, salivary expression of the HER2 receptor may not be a reliable alternative to tissue assessment.

Keywords: breast cancer, HER2, saliva, diagnosis

Introduction

HER2 (human epidermal growth factor receptor 2) is a transmembrane growth receptor protein encoded by a proto-oncogene on chromosome 17q21.[1] HER2 consists of an extracellular binding domain rich in cysteines, a lipophilic transmembrane domain, and an intracellular domain with kinase activity.[2,3] Studying this protein is important for many reasons. HER2 is overexpressed in 20%–25% of breast cancers,[4] gene amplification has been observed in 30% of metastatic breast cancers,[5] and it is associated with a poor prognosis and resistance to certain chemotherapeutic agents.[6,7] Moreover, targeting the HER2 protein could reduce the pathogenicity caused by overexpression of the gene.[8,9] Trastuzumab (a recombinant humanized monoclonal antibody directed against the extracellular domain of HER2) and lapatinib are the two agents approved to treat invasive breast cancer with positive HER2 status.[5,10] When selecting patients for targeted anti-HER2 therapy, the status of the protein can be determined by two methods, ie, immunohistochemistry (to assess the HER2 protein level in the tumor) and fluorescence in situ hybridization (for HER2 gene amplification).[11] The high cost of targeted therapy and the risk of toxicity mean

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that unequivocal determination of positive HER2 status is necessary. Further, there has been considerable research interest in the HER2 extracellular domain found in biological fluids such as serum and saliva, with studies suggesting that its use may be promising for monitoring metastatic breast cancer.[12,13] The extracellular domain of HER2 is released in blood after cleavage by ADAM (disintegrin and metalloproteinase) and can be determined using immunoenzymatic reaction techniques such as enzyme-linked immunosorbent assay (ELISA). Using this technique, a strong association was observed between the serum concentration of the soluble fragment of HER2 and its status in mammary tissues.[14,15] The potential use of saliva as a diagnostic medium for breast cancer has also been discussed by some authors,[13] on the basis of a positive correlation between saliva and serum in HER2 quantification.[13] Consequently, assessment of the HER2 soluble fragment in saliva may be an alternative to invasive tissue analysis.[16]

The aim of this study was to assess the relationship between the saliva concentration of the soluble fragment of HER2 and its status in mammary tissues by anatomopathological methods.

Patients and methods

Study design

This case-control study was done in 27 patients diagnosed with breast cancer but no visible metastatic disease at the gynecology service, Maternity Souissi Hospital, Rabat, Morocco. The study protocol was approved by the local ethics committee for biomedical research in Morocco. Before entry to the study, all patients were required to sign a consent form and asked to answer a brief patient questionnaire concerning risk factors for breast cancer, eg, age of menarche, age at first birth, number of children, breastfeeding, tobacco use and alcohol intake, menopausal status, family history, and use of hormone replacement therapy and oral contraception.

Selection criteria

Patients with breast cancer were included if they were candidates for mastectomy, if they had undergone mastectomy, if they were receiving their first session of chemotherapy, and if they had no metastatic disease. Selection of cases and controls was based on HER2 status determined by immunohistochemistry as documented in medical records. Two groups were selected, ie, ten patients with HER2-positive status (score 3+) and 17 patients with HER2-negative status (scores 0 and 1+).[17]

Patients treated with trastuzumab or/and lapatinib were excluded, as were those with undetermined HER2 status or an equivocal HER2 score (2+).

Saliva collection

Saliva stimulated by chewing flavored sugar-free gum purchased from a local store was collected in the morning. All patients were asked not to eat, drink, or smoke for at least 2 hours before the test.[18,19] Each patient was required to rinse her mouth several times and to sit for 5 minutes before collection of 5 mL of stimulated saliva in a plastic cup. The saliva samples were centrifuged at 2,000 rpm for 10 minutes; the supernatant was then stored at −80°C for later determination of HER2 concentration.

Determination of HER2 concentration

The salivary HER2 protein concentration was assessed using an ELISA kit (RayBiotech, Norcross GA, USA) designed to quantify serum HER2 levels. The protein was detected by sandwich reaction, whereby protein is trapped between two antibodies, the first one is attached to ELISA solid phase and the second recognizes the HER2 extracellular domain. After incubation, the ELISA plate was washed and antibodies HRP-conjugated streptavidin is added to each well. Then the plate was washed after incubation and TMB substrate solution is pipetted to wells. The intensity of the color developed is proportional to the amount of bound HER2. The reaction was then stopped and absorbance of each well was measured at 450 nm. The unknown HER2 concentration in the saliva samples was determined from the standard curve run after each test.

Statistical analysis

The statistical analysis was performed using Statistical Package for the Social Sciences version 13.0 software (SPSS Inc., Chicago, IL, USA). The HER2 protein concentration is expressed as the median and interquartile range, and age and body mass index are expressed as the mean and standard deviation. Demographic data, ie, age of menarche, age at the first birth, number of children, breastfeeding, adverse lifestyle habits, menopausal status, and hormone use are expressed as numbers and percentages. The clinical data, including histological type, tumor size, lymph node involvement, Scarff–Bloom–Richardson (SBR) grade, hormone receptor status, and history of vascular emboli are also expressed as numbers and percentages. The cases and controls were compared using the Mann–Whitney U test. A P-value <0.05 was considered to be statistically significant.

Results

The patient demographic data are summarized in Table 1, and show that 63% of patients started menstruating after the age of 13 years, 44.4% gave birth before the age of 25 years, 37.03% had 3–4 children, and 81.4% had breastfed their children. None of the patients smoked or drank alcohol, 25.9% were postmenopausal, and 11.1% had breast cancer antecedents. None of the patients used hormone replacement therapy during menopause; however, 70.4% had previously used oral contraceptives.

The clinical data are summarized in Table 2. No statistically significant differences were found between patients with positive and negative HER2 status with regard to age, body mass index, histological type of cancer, tumor size, lymph node status, SBR grade, estrogen receptor status, or history of vascular emboli ($P>0.05$). However, a statistically significant difference in the progesterone receptor was found between the two groups of patients ($P=0.047$).

Comparison of the salivary HER2 concentration between the HER2-positive and HER2-negative breast cancer patients was not statistically significant ($P=0.45$, Figure 1).

Discussion

Our findings did not reveal a statistically significant difference in salivary HER2 protein concentration between cases and controls. Clinical characteristics collected from medical records (ie, age, body mass index, histological type of cancer, tumor size, lymph node involvement, SBR grade, estrogen and progesterone hormone receptor status, and history of vascular emboli) were compared between patients with positive and negative HER2 status, and no

Table 1 Demographic data based on participant questionnaire

	Patients (n=27)
Age of menarche, years, n (%)	
<12	1 (3.7)
12–13	9 (33.3)
>13	17 (63.0)
Age of first birth, years n (%)	
<25	12 (44.4)
25–29	8 (29.6)
>30	2 (7.4)
Children, n (%)	
0	5 (18.5)
1–2	8 (29.6)
3–4	10 (37.03)
>4	4 (14.8)
Breastfeeding, n (%)	22 (81.4)
Adverse lifestyle habits, n (%)	
Smoking	0
Alcoholism	0
Menopause status, n (%)	7 (25.9)
Family history, n (%)	3 (11.1)
Hormone use, n (%)	
Oral contraception	19 (70.4)
Hormone replacement therapy	0

Table 2 Clinicopathological features of patients

	Patients with HER2(+) status (n=10)	Patients with HER2(−) status (n=17)	*P*-value
Age, years (mean ± SD)	43±5.79	46.82±10.05	0.28
Body mass index (mean ± SD)	27.46±4.99	25.53±4.69	0.32
Histological type, n (%)			1.00
Invasive ductal carcinoma	10 (100)	15 (88.2)	
Papillary carcinoma	–	1 (5.9)	
In situ papillary carcinoma	–	1 (5.9)	
Tumor size, n (%)			0.235
T1	4 (40.0)	2 (11.8)	
T2	3 (30.0)	10 (58.8)	
T3	3 (30.0)	5 (29.4)	
Regional lymph node involvement, n (%)			0.921
N0	4 (44.4)	9 (56.3)	
N1	3 (33.3)	4 (25.0)	
N2	1 (11.1)	2 (12.5)	
N3	1 (11.1)	1 (6.3)	
SBR grade, n (%)			1.00
I	0	1 (5.9)	
II	6 (60.0)	10 (58.8)	
III	4 (40.0)	6 (35.3)	
Hormone receptors, n (%)			
Progesterone receptor (+)	6 (60.0)	16 (94.1)	0.047
Estrogen receptor (+)	5 (50.0)	14 (82.4)	0.102
Vascular emboli (+) n (%)	1 (12.5)	4 (36.4)	0.338

Abbreviations: SD, standard deviations; SBR, Scarff–Bloom–Richardson.

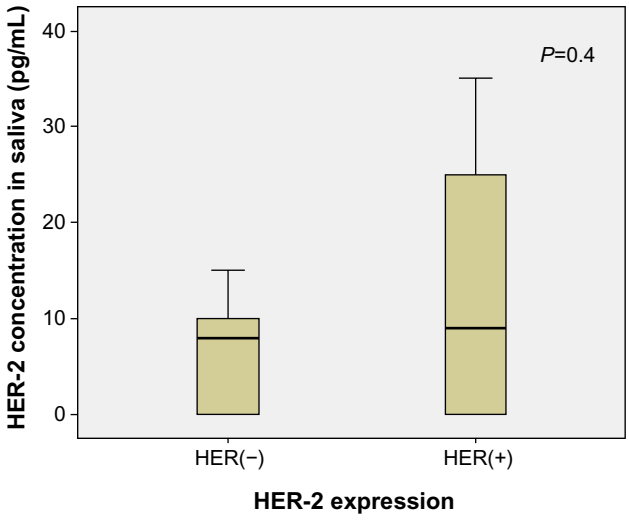

Figure 1 Salivary HER2 concentration according to positive and negative HER2 expression in mammary tissues.

statistically significant difference was found between cases and controls, except for progesterone receptor expression. Many authors have reported a negative association between progesterone receptor expression and HER2 protein expression. In one report, overexpression of HER2 protein was strongly associated with negative estrogen and progesterone receptor status.[20] Konecny et al and Mostafa et al also found an inverse association between steroid (estrogen and progesterone) receptor expression and HER2 expression,[21,22] and Badzek et al reported an association between serum HER2 and certain clinical features, including hormone receptor status. However, no relationship with histological overexpression has been observed.[23] Otherwise, there are many studies in the literature reporting reliable use of the soluble fragment of the HER2 protein in breast cancer monitoring.[24] Assessment of serum HER2 using immunoenzymatic reaction methods such as ELISA is relatively rapid, and can be repeated many times during treatment.[24] It can also be very useful for selecting patients eligible for targeted therapy and monitoring their response to this targeted therapy (trastuzumab) or response to chemotherapy.[24–28] Further, comparison of serum HER2 concentration according to receptor expression in mammary tissues has shown a significant association between HER2 status in tumor tissue and serum HER2 concentrations in many studies.[29–31] In addition, some authors have reported a strong association between serum HER2 concentration and receptor expression in mammary tissues.[14,15]

Developing a noninvasive sampling method for soluble HER2 protein in saliva would be highly desirable. Whole saliva contains a mixture of secretions from the salivary gland along with other constituents from the gingival crevicular fluid,[32,33] which is essentially serum exudate.[33] Thus, whole saliva reflects the constituents of serum and could have diagnostic potential in the detection of a number of biomarkers.[34] Saliva is a perfect diagnostic medium, and this assay has many advantages.[35,36] Sampling of saliva is technically easier than collection of blood, particularly when volunteers are invited for repeat sample collection.[35,36] Otherwise, quantifying the soluble fragment of HER2 protein in saliva is possible.[13] Streckfus et al suggested the diagnostic potential of HER2 and the possible use of saliva as a diagnostic medium based on the finding of positive and moderate correlations.[13] However, the groups compared in their study were different from the groups chosen for our study, ie, patients with positive and negative HER2 status in mammary tissue. Their study was conducted in three groups, ie, healthy women, patients with benign breast tumors, and patients with breast cancer. Moreover, the comparison was based on biological fluids, ie, serum and saliva, and the tissue status of HER2 was not considered in patients with breast cancer in their study.[13]

Studies in patients with oral squamous cell carcinoma have yielded results consistent with our data. Bernardes et al reported that salivary HER2 levels were not elevated in patients with oral squamous cell carcinoma.[37] Moreover the clinicopathological data, such as patient age, smoking habit, histological grading, T status, or nodal involvement of the tumor, the difference between patients with positive and negative HER2 overexpression in tumors was not statistically significant.[37] No differences in salivary levels was remarked in patients with positive or negative HER2 status in tumors.[37] Similar data were reported by Pardis et al, ie, salivary HER2 levels in patients with head and neck squamous cell carcinoma were not significantly higher than in healthy subjects, and there was no association between salivary HER2 levels and clinicopathological features such as age, sex, tumor grade, tumor size, or nodal status.[38]

The main limitation of our study is its small sample size, and this is attributed to the tissue determination of HER2 status, ie, many patients had an equivocal score (+2) by immunohistochemistry, so could not be included. This should encourage other studies with important sample size. In addition, the salivary concentrations should be compared to the assessment of the receptor HER2 in tumors.

In conclusion, our findings indicate that salivary expression of the HER2 receptor may not be a reliable alternative to tissue assessment because no association between salivary and tissue expression was found.

Disclosure

The authors report no conflicts of interest in this work.

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